

WHAT IS CLAIMED IS:

- 1 1. A nucleic acid molecule comprising:
2 an RNA polymerase III promoter sequence;
3 a short RNA encoding sequence comprising a transcription initiation site;
4 a STOP cassette comprising an RNA polymerase III-specific termination sequence, a
5 first *loxP* sequence and a second *loxP* sequence, wherein the *loxP* sequences flank the
6 termination sequence, and wherein the termination sequence is disposed between the
7 promoter sequence and the transcription initiation site of the short RNA encoding sequence
8 in the nucleic acid molecule; and
9 optionally the short RNA encoding sequence overlaps with one of the *loxP*
10 sequences.
11
12 2. The molecule of claim 1, wherein the first *loxP* sequence is a wild-type *loxP*
13 sequence.
14
15 3 The molecule of claim 1 or 2, wherein the second *loxP* sequence is a mutant
16 *loxP* sequence.
17
18 4. The molecule of claim 3, wherein:
19 the second *loxP* sequence overlaps some or all of the short RNA encoding sequence;
20 and
21 the n-terminal nucleotides in the terminus of the second *loxP* that is proximal to the
22 short RNA encoding sequence consists of the 5' terminal sequence of the short RNA
23 encoding sequence, wherein n=1 to 10.
24
25 5. The molecule of any one of claims 1-3, further comprising:
26 a thymidine nucleotide immediately preceding the upstream terminal sequence of the
27 first *loxP*, wherein the first *loxP* is upstream of the termination sequence.
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29 6. The molecule of any one of claims 1-5, wherein:
30 the RNA polymerase III promoter sequence comprises genomic sequence of the small
31 nuclear RNA U6 promoter or a functional equivalent thereof.

32
33 7. The molecule of claim 6, wherein:
34 the termination sequence comprises genomic sequence downstream of the small
35 nuclear RNA U6 transcription termination signal.

36
37 8. The molecule of claim 7, wherein the termination sequence is a modified U6
38 transcription termination sequence comprising:
39 some number from 1 to 20, inclusive, of additional thymidine nucleotides disposed
40 immediately adjacent to the wild-type U6 thymidine termination signal; and
41 some number from 1 to 190, inclusive, of nucleotides encoding the wild-type U6
42 genomic sequence that is immediately downstream of the thymidine termination sequence.

43
44 9. The molecule of claims 7 or 8, wherein the termination sequence further
45 comprises:
46 one or more additional RNA Polymerase III termination signals.

47
48 10. The molecule of any one of claims 1-9, wherein:
49 the short RNA encoding sequence encodes a transcript with fewer than 30
50 nucleotides.

51
52 11. A transgenic animal whose genome comprises the nucleic acid molecule of
53 any one claims 1-10.

54
55 12. The transgenic animal of claim 11, further comprising a nucleic acid molecule
56 encoding a Cre recombinase.

57
58 13. The transgenic animal of claim 12, wherein expression of the Cre recombinase
59 is developmentally regulated.

60
61 14. The transgenic animal of claim 11, wherein expression of the Cre recombinase
62 is tissue-specific.

63
64 15. The animal of any one of claims 10-14, wherein the animal is any one of the
65 following: a mouse, a rat, a guinea pig, a goat, a pig, a monkey, a baboon, a chimpanzee, a
66 cow; a rabbit; a sheep, dog, a cat, a hamster, a chicken, a frog.

67
68 16. A eukaryotic cell comprising the nucleic acid molecule of any one of claims
69 1-10.

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71 17. The cell of claim 16, wherein the cell is an animal cell.

72
73 18. The cell of claim 16, wherein the cell is a mammalian cell.

74
75 19. The cell of claim 17 or 18, wherein the cell is an embryonic stem cell.

76
77 20. The cell of any one of claims 16-19, further comprising a nucleic acid
78 molecule encoding a Cre recombinase gene.

79
80 21. The cell of any one of claims 16-19, further comprising a Cre recombinase
81 protein.

82
83 22. A method of making an inducible short RNA expression system, the method
84 comprising:

85 linking two or more nucleic acids to produce the nucleic acid of any one of claims 1-
86 10.

87 23. A method of making a transgenic animal comprising:
88 introducing the molecule of any one of claims 1-10 into the genome of an embryonic
89 stem cell;

90 introducing the embryonic stem cell into an embryo;

91 implanting the embryo in an animal capable of carrying the embryo to term; and
92 allowing the embryo to come to term, thereby generating a transgenic animal.

93
94 24. The method of claim 23, wherein:
95 the molecule of any one of claims 1-10 is introduced into the genome of an oocyte;
96 the oocyte is fertilized to produce an embryo;
97 the embryo is implanted in an animal capable of carrying the embryo to term; and
98 the embryo is allowed to come to term, thereby generating a founder transgenic
99 animal.

100
101 25. The method of claim 23, wherein the method generates a chimeric transgenic
102 animal, and further comprising:
103 crossing the chimeric transgenic animal to another animal of the same species to
104 generate a founder transgenic animal whose genome includes the molecule of any one of
105 claims 1-10.

106
107 26. A method of making an animal cell containing an inducible short RNA
108 expression, the method comprising:
109 transfecting a cell with the molecule of any one of claims 1-10.

110
111 27. The method of claim 26, wherein the cell is a cell from any one of the
112 following animals: a human, a mouse, a rat, a guinea pig, a goat, a pig, a monkey, a baboon,
113 a chimpanzee, a cow; a horse, a rabbit; a sheep, a chicken, a dog, a cat, a frog, or a fish.

114
115 28. A method of studying gene function in a cell, the method comprising:
116 providing the cell of any one of claims 16-21;
117 inducing transcription of the short RNA encoding sequence; and
118 monitoring changes in the cell.

119
120 26. A method of studying gene function in an organism, the method comprising:
121 providing the transgenic animal of anyone of claims 11-15;

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122 inducing transcription of the short RNA encoding sequence; and
123 monitoring changes in the organism.
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